Claims

1. Method for HLA typing by the unambiguous determination of short DNA sequence elements (2-6 bases) at a given position simultaneously on both parental alleles at a selected number of positions in HLA genes, comprised of the steps for each position of a) hybridising a combination of oligonucleotides (primers) complementary to all known sequence variants to a DNA strand upstream of a given position; b) carrying out a primer extension reaction with at least one of the four dNTP substrates substituted by a terminating analog; c) analysing the products by mass spectrometry, with the resulting masses allowing unambiguous identification of the used primers and the added bases.
2. Method according to claim 1 where the DNA strand of step a) is produced by a DNA replication procedure such as PCR or rolling circle replication.
3. Method according to claim 1 where the combination of primers has slightly varying sequences so that all sequences of the haplotypes are represented by a perfectly matching primer.
4. Method according to claim 3 where mass shifting tags are added to the individual primers sequences to make them uniquely distinguishable once the terminating base is added.
5. Method according to claim 1 where distinguishable termination products for known alleles are generated by extending the perfectly hybridised primer with a combination of dNTPs and ddNTPs or analogs thereof with a DNA polymerase to generate specific termination products.
6. Method according to claim 1 where the GOOD assay is used.
7. Method according to any of the precedent claims where mass spectrometry, in particular MALDI or ESI mass spectrometry is used for analysis of the masses of products.
8. Method for HLA typing according to any of the precedent claims above where set of multiple selected positions are queried to achieve sufficient information content.
9. Method for HLA typing of HLA-A according to claims 1-8 where assays of the positions 98, 414, 539, 282, 571, 368, 256, 292, 238, 270, 453, 527, 502, 81,

268, 559, 92, 123 and 396 (according to the numbering of the HLA-A gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.

10. Method for HLA typing of HLA-B according to claims 1-8 where assays of the positions: 539, 419, 559, 412, 272, 362, 302, 363, 206, 369, 259, 97, 583, 292, 222, 527, 418, 435 and 571 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.

11. Method for HLA typing of HLA-DRB1 according to claims 1-8 where assays of the positions 125, 196, 197, 227, 261, 286, 299, 308, 341 and 345 (according to the numbering of the HLA-DRB1 gene starting at cDNA sequence position 1 of exon 1) are used to achieve medium resolution.

12. Method for HLA typing of HLA-A according to claims 1-8 where assays of the positions 98, 414, 539, 282, 571, 368, 256, 292, 238 and 270 (according to the numbering of the HLA-B gene starting at cDNA sequence position 1 of exon 1) are used to generate subgroups A-O.

13. Method for HLA typing according to claim 12 where assays of the positions 224, 268, 376, 502, 561 and 616 are preferably analysed to resolve subgroup HLA-A_A; positions 126 and 526 to resolve subgroup HLA-A_B; positions 81, 90, 92, 212, 214, 257, 265, 299, 302, 404, 420, 427, 453, 485, 489 and 502 to resolve subgroup HLA-A_C; positions 160, 200, 362 and 524 to resolve subgroup HLA-A_D; positions 180, 299, 301, 302, 346, 418, 453, 517, 524, 526, 527, 557, 559 and 560 to resolve subgroup HLA-A_E; positions 299, 301, 302, 341 and 583 to resolve subgroup HLA-A_F; positions 127, 341, 399, 480, 502, 503, 524, 526, 527, 553, 559, 560 and 565 to resolve subgroup HLA-A_G; positions 228, 233, 463, 519, 530 and 583 to resolve subgroup HLA-A_H; positions 102, 275, 317, 362, 418, 419, 497, 524, 555, 595 and 618 to resolve subgroup HLA-A_I; positions 92, 331, 453, 524, 559, 560 and 564 to resolve subgroup HLA-A_J; positions 78, 81, 123, 125, 142, 144, 194, 268, 294, 324, 355, 362, 396, 403, 419, 453, 456, 477, 493, 517, 524, 526, 527, 559 and 560 to resolve subgroup HLA-A_K; positions 113, 299, 301, 302, 308, 311, 523, 524 to resolve subgroup HLA-A_L; positions 171, 363, 498 and 559 to resolve

subgroup HLA-A_M; positions 376, 426, 527, 555, 557 and 595 to resolve subgroup HLA-A_N; position 299 to resolve subgroup HLA-A_O are used.

14. Method for HLA typing of HLA-B according to claims 1-8 where assays of the positions 539, 419, 559, 412, 272, 362, 302, 363, 206 and 369 (according to the numbering of the HLA-B gene starting at DNA sequence position 1 of exon 1) are used to generate subgroups A-AC.

15. Method for HLA typing according to claim 14 where assays of the positions 259, 341 and 473 are preferably analyzed to resolve subgroup HLA-B_A; positions 106, 144, 222, 259, 273, 311, 313, 418, 445, 493, 528 and 540 to resolve subgroup HLA-B_B; positions 319, 416, 545 and 572 to resolve subgroup HLA-B_C; positions 106, 131, 165, 215, 243, 277, 292, 322, 481, 582, 603 and 616 to resolve subgroup HLA-B_D; positions 106, 146, 165, 181, 238, 259, 263, 292, 328.1/329, 379, 435, 453, 463, 485, 526, 571, 572 and 583 to resolve subgroup HLA-B_E; positions 142, 171, 255, 257, 395, 430, 544, 566 and 572 to resolve subgroup HLA-B_F; positions 117, 247, 248, 277, 345, 418, 489 and 527 to resolve subgroup HLA-B_G; positions 134, 141, 200, 213, 259, 304 and 527 to resolve subgroup HLA-B_H; positions 83, 141, 211, 222, 242, 322, 404, 414, 435, 463, 502, 527, 544, 571, 572 and 583 to resolve subgroup HLA-B_I; positions 103, 142, 222, 243, 259, 292, 477, 486 and 499 to resolve subgroup HLA-B_J; positions 103, 259, 292, 295, 527 and 583 to resolve subgroup HLA-B_K; positions 320 and 500 to resolve subgroup HLA-B_L; positions 311, 527 and 583 to resolve subgroup HLA-B_M; positions 119, 292, 259, 319, 425, 527, 546 and 583 to resolve subgroup HLA-B_N; positions 97, 142, 245 and 527 to resolve subgroup HLA-B_O; positions 97 and 175 to resolve subgroup HLA-B_P; positions 246 and 277 to resolve subgroup HLA-B_Q; positions 246, 292, 311 and 503 to resolve subgroup HLA-B_R; positions 103, 261, 309, 311 and 474 to resolve subgroup HLA-B_S; positions 97, 103, 106, 243, 259, 292, 404 and 524 to resolve subgroup HLA-B_T; positions 259 and 320 to resolve subgroup HLA-B_U; position 106 to resolve HLA-B_V; positions 97 to resolve HLA-B_W; positions 97, 106, 257, 418 and 463 to resolve HLA-B_X; position 106 to resolve HLA-B_Y; positions 106 and 144 to resolve HLA-B_Z; positions 117, 247, 248, 283, 345, 418, 489, and 527 to

resolve HLA-B_AA; positions 106 to resolve HLA-B_AB; positions 548 to resolve HLA-B_AC .

16. Method of HLA typing according to claim 11 to resolve subgroups A-P of HLA-DRB1.

17. Method for HLA typing according to claim 16 where assays of the positions 123, 174, 250, 278 and 317 are analysed to resolve subgroup HLA-DRB1_A; positions 192, 203, 256 and 259 to resolve subgroup HLA-DRB1_B; 256, 260, 317 and 351 to resolve subgroup HLA-DRB1_C; positions 155, 204, 233, 239, 256, 304, 357 and 366 to resolve subgroup HLA-DRB1_D; positions 122, 171, 257 and 317 to resolve subgroup HLA-DRB1_E; positions 164, 167, 171, 230, 235, 306, 317, 321 and 337 to resolve subgroup HLA-DRB1_F; positions 164, 257, 266 and 303 to resolve subgroup HLA-DRB1_G; positions 164, 181, 188, 220, 229, 256, 266, 317 and 318 to resolve subgroup HLA-DRB1_H; position 257 to resolve subgroup HLA-DRB1_I; positions 181, 239 and 357 to resolve subgroup HLA-DRB1_J; positions 122, 144, 239, 303, 317, 318 and 321 to resolve subgroup HLA-DRB1_K; positions 118, 161, 257, 260, 318 and 321 to resolve subgroup HLA-DRB1_L; positions 165, 257, 293 and 303 to resolve subgroup HLA-DRB1_M; positions 177, 240, 256, 257 and 357 to resolve subgroup HLA-DRB1_N; positions 150 175, 230, 236 and 321 to resolve subgroup HLA-DRB1_O; positions 115, 220 and 317 to resolve subgroup HLA-DRB1_P are used.

18. Kit for the implementation of the procedure according to claims 1 - 17 comprising pools of primers.

19. Use of the method according to claims 1-17 for screening of tissue donors.

20. Use according to claim 19 for bone marrow donors in registries for screening of frequent and rare HLA types.

21. Use of the primers represented in Table IV, V and VI to carry out HLA typing.